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Recent Progress in the Transgenic Modification of Swine and Sheep

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INTRODUCTION

For centuries the genetic composition of domestic animals has been manipulated to enhance their usefulness to man. In the past decade, development of recombinant DNA technology has enabled scientists to isolate single genes, analyze and modify their nucleotide structures, make copies of these isolated genes, and transfer copies into the genome. Animals that integrate this recombinant DNA in their genome are called "transgenic." Recently, medically important human proteins have been produced in large quantities in milk of transgenic sheep. Unless unforeseen complications arise during extraction and purification of these proteins, we can expect to see such products to begin clinical evaluation very soon.

Use of transgenic animals for food and fiber remains more distant in the future. Few agriculturally useful genes have thus far been isolated, sequenced, and cloned. In addition, insufficient information on gene regulation is available so that these integrated transgenes can be regulated sufficiently to prevent overexpression that has been shown in some instances to adversely impact the health status.

The purpose of this paper will be to briefly review the progress that has been achieved since transgenic modification of swine and sheep was first reported in 1985 (Hammer et al., 1985), with emphasis on developments during the past year.

GENE TRANSFER METHODS

The predominant method used to transfer cloned genes into animals is direct microinjection into the pronuclei of fertilized eggs. Although microinjection of mouse pronuclei is readily performed, direct application to other species was impeded by the opacity of the egg cytoplasm. Pronuclei of sheep eggs can be readily seen using differential interference contrast (DIC) microscopy. However, pig and cow eggs must be centrifuged at 15,000*g* for 5 min to induce stratification of the cytoplasm before the pronuclei are visible with use of DIC microscopy (Wall et al., 1985).

The efficiency of transgene integration tends to be lower for farm animals than for mice. The percentage of gene-injected embryos that develop into transgenic animals varied from 0.3 to 4.0% for pigs and 0.1 to 4.4% for sheep. Some of the important parameters that influence the frequency of integration have been described for mice (Brinster et al., 1985) but have not been systematically investigated in other species.

TRANSGENES FOR PRODUCTIVITY TRAITS

Transfer of genes for improved animal productivity traits, such as feed conversion; rate of gain; reduction of fat; and improved quality of meat, milk, or wool, would have a dramatic impact on the livestock industry and reduce the cost of animal products for the consumer. These productivity traits are controlled by numerous genes, only a few of which are presently known.

Growth Hormone (GH)

Chimeric genes integrated into genomes of pigs contained either metallothionein (MT), phosphoenolpyruvate carboxykinase (PEPCK), viral LTR (MLV and CMV), prolactin (PRO), albumin (AL), or transferrin (TF) regulatory (enhancer/promoter) sequences fused to the structural sequences encoding bovine growth hormone (bGH), human growth hormone (hGH), porcine growth hormone (pGH), or human growth hormone releasing factor (hGRF) (Hammer et al., 1985; Brem et al., 1985; Pursel et al., 1987, 1989, 1990b; Wieghart et al., 1990; Polge et al., 1989; Ebert et al., 1990; Vize et al., 1988). Also, MT-bGH, TF-bGH, MT-hGH, ovine GH (oGH), MT-hGRF, and AL-hGRF have been transferred into sheep (Hammer et al., 1985; Rexroad et al., 1989, 1991; Murray et al., 1989). Most of the transgenic pigs and lambs that expressed these transgenes had continuously elevated GH in their plasma. The concentration of GH varied greatly among transgenic with the same structural gene, which is thought to be the result of transgenic insertion at random in the genome. In general, pigs and lambs did not grow larger than their siblings. Some pigs gained weight faster, were 17% more efficient in converting feed into meat, and at market weight contained only one-fifth as much carcass fat as littermates (Pursel et al., 1990b). Transgenic lambs did not grow faster or utilize feed more efficiently than control lambs, but they were much leaner. In transgenic lambs, the lack of body fat may have been the result of hyperglycemia and glycosuria (Rexroad et al., 1989, 1991), GH transgenic

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pigs with continuously elevated GH had a high incidence of joint pathology, gastric ulcers, and infertility (Pursel et al., 1987, 1989; Ebert et al., 1988, 1990; Weighart et al., 1990).

In transgenic mice, both MT and PEPCK regulatory sequences could be manipulated by dietary changes to modify expression of GH transgenes, but these regulatory sequences were much less responsive to dietary manipulation in transgenic pigs and sheep (Pursel et al., 1990a; Weighart et al., 1990; Murray et al., 1989). Fully regulatable GH transgenes could provide positive effects of elevated GH during the latter portion of the growth period. In the future, such a high degree of regulated transgene expression should be possible.

Stimulation of Muscle Development

Sutrave et al. (1990) reported that mice expressing a chicken cSKI transgene exhibited a distinct phenotype characterized by hypertrophy of skeletal muscles and reduced body fat. The gene transferred into mice consisted of a mouse sarcoma virus (MSV) LTR promoter fused to a truncated cSKI cDNA. The transgene product is a protein containing 448 amino acids that is localized primarily in muscle nuclei. The normal function of cSKI and its mode of action is unknown.

The MSV-cSKI gene has now been transferred into the genome of swine (Pursel et al., 1992). Preliminary results indicate that expression of the cSKI transgene in swine resulted in a wide range of phenotypic responses among animals. Five transgenic pigs exhibited varying degrees of muscular hypertrophy that was visually detected around 3 months of age. In three pigs, both hams and shoulders appeared enlarged, while in two pigs, hypertrophy was evident only in the shoulders. Levels of gene expression in muscles of these pigs have not yet been determined. In contrast, between birth and 3 months of age, five other cSKI transgenic pigs exhibited muscular atonia and weakness in both the front and rear legs. Skeletal muscles from these pigs had high levels of cSKI mRNA, while cardiac muscle contained low levels, and no transgene mRNA was detected in any other tissue. Histological examination of skeletal muscles from these myopathic pigs revealed that muscle fibers contained large vacuoles. None of the cSKI transgenic mice exhibited the myopathic phenotype found in pigs. However, one hypertrophic line of cSKI mice had centrally located nuclei in some muscle fibers, and other fibers contained small vacuoles (S.H. Hughes, P. Sutrave, and A.M. Kelly, personal communication).

Muscle phenotype was unaltered in the other cSKI transgenic pigs, seven of which were biopsied and cSKI mRNA evaluated. Northern transfer revealed cSKI expression in biceps femoris of three pigs, in semimembranosus of four pigs, and in triceps brachii of five pigs. More complete characterization of the cSKI transgenic pigs will be made with progeny of these founders when they become available.

Immunoglobulin A (IgA)

Genes encoding mouse α heavy and κ light chains from antibodies against phosphorylcholine (PC) were co-injected into ova to produce two transgenic pigs and three transgenic lambs (Lo et al., 1991). In the transgenic pigs, the mouse IgA was detected in the serum despite the failure of an intact mouse κ transgene to integrate. However, mouse IgA showed little binding specificity for PC, presumably because secreted antibody included endogenous pig light chains. In transgenic sheep, mouse IgA was detectable in peripheral lymphocytes but not in serum. These studies need to be expanded to obtain conclusive proof that the IgA transgene would be protective against pathogenic bacteria.

DIRECTING TRANSGENE EXPRESSION TO MAMMARY GLANDS

The most significant recent advance in gene transfer involves the direction of expression of considerable quantities of foreign protein to the mammary glands of sheep and swine. A key factor responsible for this high level of transgene expression is the use of genomic DNA for the structural gene instead of cDNA, which had been used previously. These findings fully support the earlier research of Brinster et al. (1988) in mice that showed inclusion of introns in gene constructs resulted in higher levels of transgene expression than when the same regulatory sequences were ligated to cDNA.

The regulatory sequences that prove to be most effective for directing high levels of pharmaceutical should also prove to be useful for modifying milk composition for agricultural purposes and vice versa. Several of these non-pharmaceutical purposes include: reduction of mastitis, alteration of casein composition for production of cheese, or production of human proteins in milk of farm animals to provide human infants with a better substitute, alteration of lactose composition, and reduction or alteration in butterfat content (Jimenez-Flores and Richardson, 1988; Wilmut et al., 1990).

Whey Acidic Protein (WAP)

In an attempt to alter the milk composition in livestock, Wall et al. (1991) introduced WAP, a major mouse milk protein, into the genome of swine, which do not contain a WAP gene. Fourteen transgenic pigs containing a 7.2 kb fragment of mouse WAP genomic DNA (both regulatory and structural sequences) were born. Milk and tissues from females of six transgenic lines have been evaluated. Mouse WAP was detected in milk from all lactating sows at concentrations of more than one gram per liter, and expression of the corresponding mRNA was specific to the mammary gland in all but one line in which a low level was found in the salivary gland. Mouse WAP accounted for about 3% of the total milk proteins in the transgenic sows thus demonstrating that it is possible to produce high levels of a foreign protein in pig milk.

Quite unexpectedly, five sows from three lines of WAP transgenic pigs produced milk only a few days before becoming agalactic (Shamay et al., 1991). Lacta-

tion failure was not accompanied by elevated body temperature, lack of maternal behavior, loss of appetite, nor by other evidence of illness that accompanies agalactia in swine. Neither mice nor sheep expressing other mammary-specific transgenes have previously exhibited this phenomenon. Research is in progress to determine the cause of this peculiar agalactia, which has now also been observed in several transgenic mice that are expressing the same WAP transgene earlier during gestation than normal (R.J. Wall and L. Hennighausen, personal communication).

Human Alpha-1-Antitrypsin ($h\alpha_1AT$)

Wright et al. (1991) recently reported that high levels of $h\alpha_1AT$ expression in milk have been obtained in four transgenic ewes in which sheep β-lactoglobulin regulatory sequences were ligated to $h\alpha_1AT$ genomic sequences. The concentration of $h\alpha_1AT$ at the seventh week of lactation varied from 1.5 to 37.5 g/L among the ewes, and most remarkably, $h\alpha_1AT$ made up more than half of the protein contained in the milk from one transgenic ewe (Wright et al., 1991). In spite of the extraordinarily high levels of $h\alpha_1AT$ production, the ewes had normal durations of lactation and exhibited no ill effects from the transgene. The high level of transgene expression with genomic $h\alpha_1AT$ sharply contrasted earlier investigations in which only low levels of expression were obtained when BLG had been ligated to cDNA of $h\alpha_1AT$ (Wilmut et al., 1990) or human Factor IX (Clark et al., 1989).

The glycosylation and biological activity of $h\alpha_1AT$ purified from transgenic sheep milk appeared to be indistinguishable from that of $h\alpha_1AT$ purified from human plasma (Wright et al., 1991). Whether the sugar composition of the carbohydrate side chains has been altered remains to be determined.

CONCLUSIONS

Although efficiencies in transferring genes remain low and the list of genes that are worthy candidates for enhancing the productivity of livestock is short, achievements during the past year should encourage expansion of research on genetic engineering of livestock. The success in directing high levels of transgene expression to the mammary gland should stimulate considerable activity on human pharmaceuticals and on efforts to modify milk composition. In addition, the need for a better understanding of gene regulation should stimulate additional research in that important area.

Considerable data now shows that use of genomic DNA instead of cDNA is highly advantageous for obtaining both high levels of expression and a high percentage of transgenic animals that express their transgene. Cloning of the genomic sequences is not a trivial matter for many genes, and that extra work will present a formidable challenge if obtaining high levels of expression is essential.

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